United States
Department of
Agriculture

Forest Service

Southeastern Forest
Experiment Station

Research Paper
SE-258

Ecology of the Pinewood Nematode in Southern Pine Chip Piles

L. David Dwinell

Mention of products and companies by name is solely to identify experimental materials and does not constitute endorsement by the USDA, nor does it imply approval of a product to the exclusion of others that may also be suitable.

October 1986

Southeastern Forest Experiment Station
P.O. Box 2680
Asheville, NC 28802

Ecology of the Pinewood Nematode in Southern Pine Chip Piles

L. David Dwinell
Research Plant Pathologist
Forestry Sciences Laboratory
Athens, Georgia

ABSTRACT

In wood-chip piles of southern pine species, the pinewood nematode was found mainly in fresh chips and chips located in the outer shells of piles. Chips in the interior of piles did not harbor the nematode if the temperature reached 60 °C, caused by wood-cell respiration. In naturally infested or nematode-enhanced chips, the optimum temperature range for reproduction of the pinewood nematode was 35 to 40 °C. Samples of truck shipments of chips varied in response to incubation at 40 °C. Nematode populations declined rapidly at temperatures above 45 °C. There was no marked change in population density for 21 days at temperatures of 0 to 10 °C. The nematode did not survive in chips at -20 °C for more than 3 days. In chips that had been autoclaved and infested with the bluestain fungus (*Ceratocystis* sp.), the optimum temperature for reproduction of the nematode was 35 °C. Significantly more nematodes were recovered from chips colonized by bluestain than those not colonized. Wood moisture had a marked impact on the population density of the nematode in chips. The pinewood nematode was intolerant of an anaerobic environment at 38 °C. Pinewood nematodes extracted from southern pine chips were pathogenic on 2-year-old slash pine seedlings in the greenhouse.

Keywords: *Bursaphelenchus xylophilus.* temperature. bluestain. pine wilt. slash pine. loblolly pine, pathogen.

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle (= *B. lignicolus* Mamiya & Kiyohara), and pine sawyers, *Monochamus* spp., are the primary causal agents of the pine wilt disease. Although the impact of the pine wilt complex on forests in Japan has been well documented (Mamiya 1983, 1984), the role of the disease in the United States is still being defined (Wingfield and others 1982). In most cases, pine wilt is associated with exposure to environmental stress (Mamiya 1983, 1984; Wingfield and others 1982). The disease was not considered a problem in southern pines until the pinewood nematode was discovered in dying pines in several seed orchards (Dwinell and Barrows-Broaddus 1983).

All 43 species of *Bursaphelenchus* described in the literature have a phoretic relationship with insects, especially bark beetles and wood borers, and all are mycophagous (Massey 1974; Riihm 1956; Tarjan and Aragón 1982). The pinewood nematode, however, is a facultative parasite capable of living and reproducing on both fungi and higher plants (mainly *Pinus* spp.) (Mamiya 1983, 1984). Adult pine sawyers (*Monochamus* spp.), which are wood borers, are vectors of the pinewood nematode and other species of *Bursaphelenchus.* The borers colonize pine logs held in storage or pines weakened or killed by natural or manmade stresses (Hellrigl 1971; Webb 1909). The pinewood nematode is transmitted by mature beetles feeding on shoots of stressed trees or ovipositing in dying trees or cut timber (Luzzi and others 1984; Wingfield 1983).

In 1984, the pinewood nematode was found infesting wood chips exported from North America. Finland placed a permanent embargo on raw softwood shipments from the United States, Canada, Japan, and other infested regions of the world. The other Nordic countries have followed suit. The curtailment of southern pine chip exports to Nordic countries would represent about a $20 million per year loss to southern forestry.

At the shipping terminal (Woodchip Export Co.) in Savannah, GA, chips of southern pine are accumulated in two piles; each contains 15,000 to 20,000 t when loading of an ocean vessel begins (fig. 1). Normally, it takes 6 to 8 weeks to accumulate the required tonnage of wood chips (about 35,000 t). An estimated 70 percent of the chips are from slash pine (Pinus elliottii Engelm. var. elliottii) and 30 percent are from loblolly pine (P. taeda L.) (Wayne Stubbs, personal communication).

The development of micro-organisms in the chip piles is mainly governed by temperature. The temperature in a chip pile depends on the ambient temperature, the size and compaction of the pile, and the fines and bark content of the chips (Bergman 1985). Regardless of the ambient temperature, the interior of the pile rapidly rises to 60 °C. The temperature of the outer layer, or shell of the pile, is usually below the ambient temperature (Bergman 1985; Rothrock and others 1961; Saucier and Miller 1961; Schmidt 1969; Springer and Haljny 1970). The initial spontaneous heating in piled wood chips is attributed primarily to heat released by respiration in living sapwood cells (Bergman 1985; Springer and Haljny 1970).

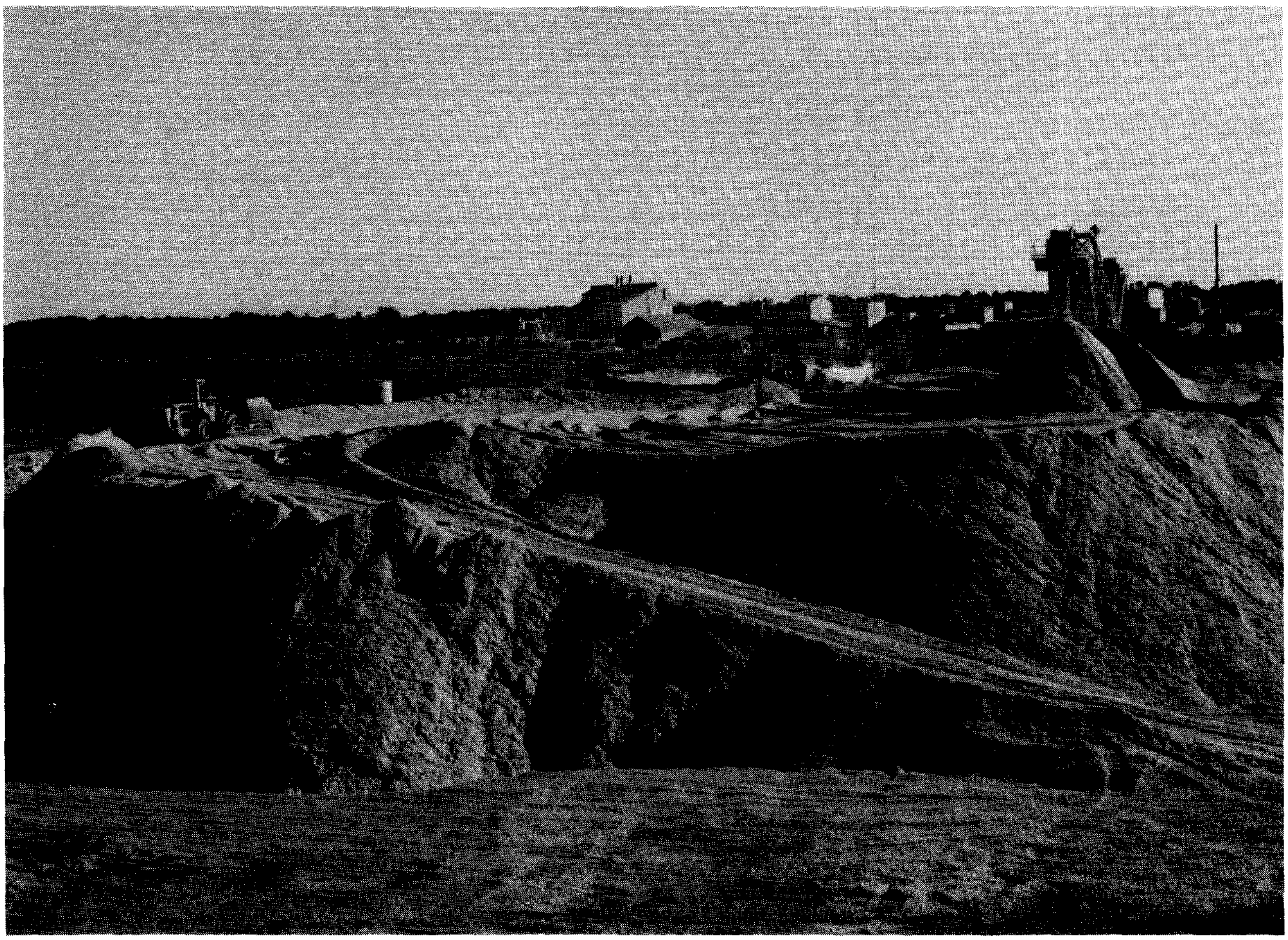

Figure 1.--Southern pine chips **(38,000 t)** ready for shipment to Sweden.

To develop strategies for controlling the pinewood nematode in southern pine chips, information is needed on the biology of the organism in this unique ecological niche. The studies described here were undertaken to determine:

- What types of chips harbor the nematode.
- Effects of temperature on the reproduction of the nematode in chips.
- Influence of bluestain fungi in chips on nematode populations.
- Influence of chip moisture on nematode population dynamics.
- Effects of anaerobic conditions on nematode survival.
- Effects of covering chip piles with plastic on the thermodynamics of the pile, as well as changes in nematode populations.
- Pathogenicity to pine seedlings.

Methods and Materials

Nematode Assay

Nematodes were extracted from chips by the pie-tin technique of Kable and Mai (1968). Samples were incubated for 18 to 20 hours at 25 °C and collected on a 325-mesh screen. The extracted nematodes were concentrated in 10 mL of deionized water. Nematodes in a 1-mL aliquot were counted under a Wild dissecting microscope and expressed as the number per gram of fresh wood weight.

In some experiments, the population densities were increased in chip lots by spraying the chips with a pinewood nematode suspension. The population of the pinewood nematode used in these experiments was originally isolated from Virginia pines (*Pinus virginiana* Mill.) in a seed orchard in Alabama. It was reared on *Botrytis cinerea* Pers. ex Fr. growing on potato-dextrose agar at 25 C. Nematodes were extracted from 7- to lo-day-old cultures by the pie-tin technique, then collected on a 325-mesh screen.

Chip types. Samples of the following chip types were screened for nematodes: (1) Fresh--Chips collected from truck or railcar loads prior to loading on the pile. A large percentage of the chips were discolored by the bluestain fungus. (2) Shell--Chips, also discolored, were collected from the outer layer of the pile. (3) Interior--Chips were collected from the interior of the pile where the temperature was 60 °C. (4) Foundation--Chips that were dark-stained, had accumulated on the ground over several years, and had served as the base for the new chip pile.

Ten 100-g samples of each of the chip types were placed in polyethylene interlock seal bags (zip-lock) and incubated at 25 °C for 2 and 8 weeks (5 samples per incubation period). In addition, ten 75-g samples of each of interior, fresh, and foundation chips were mixed with 25 g of shell chips, and incubated at 25 °C for 2 and 8 weeks. At the end of the incubation periods, the samples were assayed for nematodes. Data were subjected to split-plot analysis of variance, with time as the main plot.

Temperature. The population density of the nematodes in 6 kg of fresh southern pine chips was increased by spraying the chips with 200 mL of a suspension containing 1,250 pinewood nematodes/ml. These infested chips were mixed and stored in a plastic bag at 25 °C for 1 week. These chips were then divided into 50-g samples, placed in zip-lock bags, and incubated at 25, 30, 40, 50, and 60 °C. After 1, 2, 3, 4, and 5 days, two samples (replications) were removed from each incubator and assayed for pinewood nematodes. Data were subjected to split-plot analysis of variance procedures, with temperature as the main plot.

In a second temperature study, 6 kg of wood chips were heated in an oven at 60 °C for 24 hours, spread on a table, and sprayed with 200 mL of a suspension containing 1,000 pinewood nematodes/ml.

These infested chips were incubated at 25 °C for 2 weeks and then divided into 50-g samples placed in zip-lock bags. The samples were incubated at 25, 30, 35, 40, 44, and 48 °C. After incubation periods of 1, 3, 7, 14, and 21 days, four samples at each temperature were assayed for the pinewood nematode. Data were subjected to split-plot analysis of variance, with temperature as the main plot.

Wood chips naturally infested with the pinewood nematode were incubated for 4 days at 25 and 40 °C. Each treatment was replicated three times, and the sample size was 75 g. Aliquots of the collected nematodes were examined with a compound microscope, and the proportions of males, females, and juveniles were determined for 150 nematodes at each incubation temperature.

Fifty-g samples of 10 lots of chips were taken from truck and railcar shipments to the terminal during July 1-5, 1985. They were incubated in zip-lock bags at 25 and 40 °C for 4 days. The samples were assayed for the pinewood nematode at the end of the incubation period, and the data were compared by t-test for paired samples.

To study effects of high temperatures on the pinewood nematode, 50-g samples of naturally infested chips were placed in zip-lock bags and incubated at 50 and 60 °C for 1, 2, 3, 5, 13, and 24 hours. Each treatment combination was replicated six times. The nematode population density was determined at the beginning of the experiment by assaying three 50-g samples. The treatment samples were assayed for the pinewood nematode at the end of each incubation period. The data were not subjected to statistical analysis.

To determine the effects of low temperature on nematode density, 3.6 kg of chips--returned to the United States following a shipment to Sweden--were divided into 50-g samples. Samples were placed in zip-lock bags and incubated at -20, 0, 5, 10, 15, and 20 °C. After incubation periods of 1, 3, 7, or 14 days, three samples from each temperature were assayed for the pinewood nematode. Data were subjected to split-plot analysis of variance, with time as the main plot.

An experiment was also conducted to determine the effect of temperature on the pinewood nematode in autoclaved southern pine chips. Approximately 2.4 kg of chips were autoclaved for 45 minutes, allowed to cool, then dipped in a suspension of conidia that had been collected from cultures the bluestain fungus, <u>Ceratocystis</u> sp., grows on malt agar at 25 °C. The conidia were washed from three culture plates and suspended in 2 L of sterile deionized water. After dipping, the chips were drained and incubated in the laboratory for 3 days at 25 °C. They were spread on a plastic tray and evenly sprayed with a 100 mL suspension of pinewood nematodes (1,000/mL). Four days later, these infested chips were divided into 50-g samples, placed in zip-lock bags, and incubated at 25, 30, 35, and 40 °C for 1, 3, 6, and 9 days. There were three replications of each treatment combination. Data were subjected to two-way analysis of variance.

Bluestain. On July 1-5, 1985, a total of 38 truck and railcar shipments to the exporter were randomly sampled. Individual chips from 100-g subsamples were separated according to presence or absence of bluestain. The resulting subsamples were reweighed, and the percentage of weight by chips with or without bluestain was determined for each of the 38 lots. The samples were then readjusted so that each group had a fresh weight of 50 g. These adjusted samples were assayed for nematodes, and data were compared by the t-test for paired observations.

Ten of the 38 lots that had been divided into subsamples containing chips with or without bluestain were incubated in zip-lock bags at 40 °C for 6 days and then assayed for nematodes. The sample weight was 50 g, and each treatment combination was replicated twice. Data were subjected to two-way analysis of variance and t-test for paired observations.

Chip moisture. The influence of chip moisture on nematode population density was investigated. From a 1.2-kg sample of chips naturally infested by the pinewood nematode, 25-g samples were randomly selected and placed in tared weighing dishes. The resulting 45 samples were placed in desiccators containing calcium chloride, water, or neither (15 samples/treatment) and incubated at 35 °C. Six samples were assayed for nematodes to determine the base level. After incubation periods of 2, 5, 9, 13, and 21 days, three samples were removed from each container, weighed to determine the percentage of moisture loss, and assayed for nematodes. Data were subjected to two-way analysis of variance and regression.

Anaerobic environment. Effects of an anaerobic environment (CO_2) on nematode population densities in pine chips were studied in GasPak Plus Anaerobic Jar System (BBL Microbiology Systems, Cockeyville, MO 21030). In one experiment, 39-g samples of six randomly selected lots of chips were placed in. petri dishes, sealed in a GasPak, and incubated at 38 °C for 3 days. The control samples were concurrently incubated in an aerobic environment at the same temperature. At the end of the experiment, the samples were assayed for nematodes, and the data were compared by t-test for paired observations.

In a second experiment, the base nematode population densities were determined. Then 39-g samples of 10 randomly selected lots of chips were incubated for 5 days at 38 °C in an aerobic environment and transferred to an anaerobic environment for 3 days. Two sets of controls, incubated in an aerobic environment at 38 °C for 5 to 8 days, were included. The samples were assayed for nematodes at 5 or 8 days. Data were compared by t-test for paired observations.

Chip piles. A study was conducted to learn about thermodynamics of piled chips and the effects of heat on nematode population dynamics in chip piles. Two cone-shaped chip piles, 6 m in diameter at the base and 2.4-m height were constructed. Chips were taken from the shell of a nearby main pile in Savannah with a front loader. The piles were constructed with four layers (fig. 2). Each layer was implanted with 450 g chip samples infested with nematodes and enclosed in plastic hardware cloth. The numbers of samples for layer 1 (bottom), 2, 3, and 4 (peak) were 4, 3, 2, and 1, respectively.

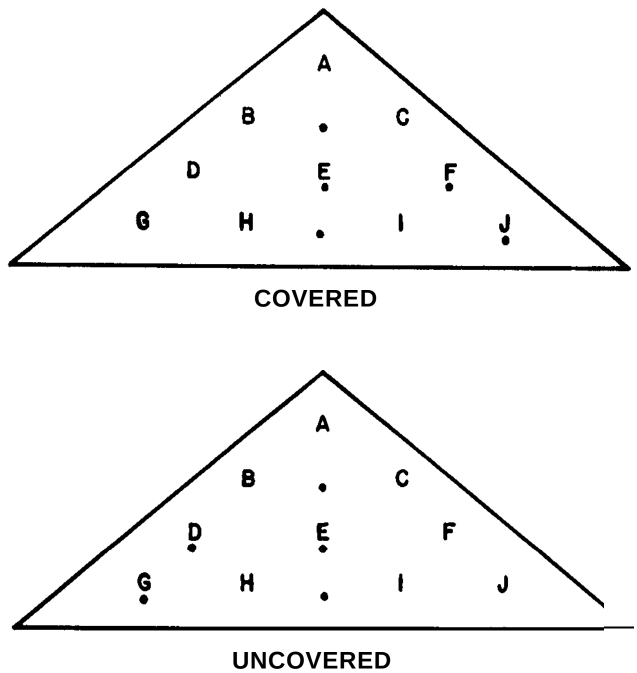

Figure 2. --Diagram of simulated chip piles showing the location of the pinewood nematode-infested chip samples (A-J) and the temperature probes (o).

Five thermistor probes were placed at different locations in the piles and connected to an analog telethermometer (Model 47, Yellow Springs Instruments, Inc., Yellow Springs, OH) housed in a standard weather station enclosure located between the piles. An eleventh probe measured ambient temperature. One of the piles was covered with a sheet of 4-mil plastic: the other was left uncovered. The temperatures were recorded Monday through Friday at 0800, 1200, and 1600 hours.

The study was established August 26, 1985, and terminated September 12, 1985. At the conclusion of the study, the chip implants were removed from the piles and assayed for nematodes by screening three 50-g subsamples from each bag. Data were compared for covered and uncovered piles and location within the pile by t-test for paired observations.

Pathogenicity. The pinewood nematodes extracted from 10 lots of chips that had been incubated for 4 days at 25 or 40 °C were used in a preliminary study on pathogenicity to 2-year-old seedlings of slash pine in a greenhouse. Seedlings were grown in plastic flats (33x13x11 cm) containing a mixture of soil, pine bark, and sand (2:1:1, v/v). Each flat contained 10 seedlings that had been transplanted when they were 1 year old.

The 20 populations used as inocula were adjusted to 1,000 nematodes/ml. Since the inocula were from naturally infested chips, other nematode species and associated organisms were not removed from the inoculum suspension. To inoculate each seedling, an area (3 mm x 10 mm) of bark on the main stem was removed, then a piece of sterile, absorbent cotton containing 200 pinewood nematodes was attached with Parafilm over each stem.wound. Five seedlings were inoculated with each nematode population. Equal numbers of wounded but uninoculated seedlings served as controls. The study was initiated in late July 1985 and concluded 16 weeks later.

At the conclusion of the experiment, seedling mortality was recorded. Then all seedlings were sampled for nematodes by extracting from 25-mm sections of the stem directly below the inoculation point and at the base of the seedling. Sections were incubated in 5 mL of water in separate test tubes at 25 °C for 20 hours. Extracted nematodes were not counted. Mortality data (25 vs. 40 °C) were compared by using the t-test for paired observations.

Results

Chip types. Most of the nematodes extracted were saprobic species rather than pinewood nematodes. An analysis of variance of the total nematode or pinewood nematode population densities showed that the experimental variation was caused by chip types or mixes and not by incubation period or treatment interactions. There was, however, a notable increase in general nematode densities in fresh chips between week 2 and week 8 of incubation (table 1). Of the four main chip types studied, nematodes were most numerous in shell and fresh chips. There were significantly ($\underline{P}$=0.01) fewer nematodes in the interior and foundation chips. The highest total nematode densities were found in the treatments having shell chips mixed with interior or fresh chips prior to incubation. Also, the nematode levels in mixtures exceeded those that would have been predicted based on the levels recorded for the individual chip types. The predicted mean nematode count for interior + shell chips was 88 nematodes/g fresh wood weight ([0.5 x 75] + [348 x 25])/100, and for fresh + shell chips it was 351 nematodes/g ([75 x 75] + [348 x 75])/100, whereas the actual count was 789 and 743 nematodes/g, respectively. Mixing shell chips with foundation chips apparently did not influence nematode population densities in the same manner, because the actual count of 66 nematodes/g was less than the predicted count of 120 nematodes/g ([57 x 75] + [348 x 25])/100.

The analysis of variance showed that population densities of the pinewood nematode in the chips were related to an interaction of chip types with incubation period. This interaction was reflected primarily in the significant ($\underline{P}$=0.01) increase in pinewood nematode counts in the fresh chips between the 2- and 8-week incubation periods and the decrease in counts in the interior + shell mix during this period. The counts in other treatments (chip types or mixes) were not affected by incubation period. Pinewood nematodes were recovered from only shell and fresh chips and were not found in interior or foundation chips.

Temperature. Temperature had a marked influence on the reproduction and

Table 1.--Assay of wood-chip types and mixes for pinewood nematode and other nematodes after incubation at 25 °C for 2 and 8 weeks

Types of wood chips and mixes[a]	All nematodes after incubation for--			Pinewood nematodes after incubation for--		
	2 wk	8 wk	Mean[b]	2 wk	8 wk	Mean
Shell (S)	130	565	348 B	14 CD	12 CD	13
Interior (I)	0.7	0.3	0.5 C	0 D	0 D	0
Fresh (FR)	30	676	353 B	5 CD	32 B	18
Foundation (FDN)	43	71	57 BC	0 D	0 D	0
I + S	789	608	698 A	55 A	9 CD	32
FR + S	743	1,034	888 A	10 CD	18 BC	14
FDN + S	51	71	66 BC	3 CD	8 CD	6

[a] Chip types: Fresh--chips collected from truck or railcar loads prior to loading on the pile. Shell--chips collected from the outer layer of the pile. Interior--chips collected from the interior of the pile where the temperature was 60 °C. Foundation--old chips that served as the base for the new chip pile. For S, I, FR, and FDN, the sample size was 100 g. The mixes consisted of 75 g of I, FR, or FDN chips plus 25-g S chips. There were five replications of each treatment combination.

[b] Within columns, means followed by the same letter do not differ significantly (P=0.01) according to Duncan's multiple range test.

survival of the pinewood nematode in these studies. In the initial experiment, the nematode population density at 40 °C increased significantly (P=0.01) over time (fig. 3). After 5 days, the population density at 40 °C was 15 times the average density for 25 and 30 °C. After 1 day, the number of nematodes in chips incubated at 50 and 60 °C was zero. At 25 and 30 °C, there was no significant change in the population densities during the period of the experiment. In naturally infested wood chips, 102 nematodes/g were extracted from samples incubated for 4 days at 25 °C and 646 nematodes/g at 40 °C. At 25 °C, 13, 17, and 70 percent of 150 nematodes examined were males, females, and juveniles, respectively. At 40 °C, 10, 29, and 61 percent were males, females, and juveniles, respectively. Only the propagative forms of the nematode were observed.

In the second experiment, the analysis of variance showed that experimental variation was influenced by the time x temperature interaction (P=0.01). At 25 and 30 °C, the nematode densities declined from 55 nematodes/g at day 1 to 23 nematodes/g at day 21 (fig. 4). At 35 °C, the population densities were 118 and 122 nematodes/g on days 3 and 7, respectively, and then declined to lower densities at later periods. At day 21, however, the nematode densities were still 2.6 times greater than those in the 25 and 30 °C treatments. The nematode densities in the 40 °C treatment increased to 250 nematodes/g by day 3 and

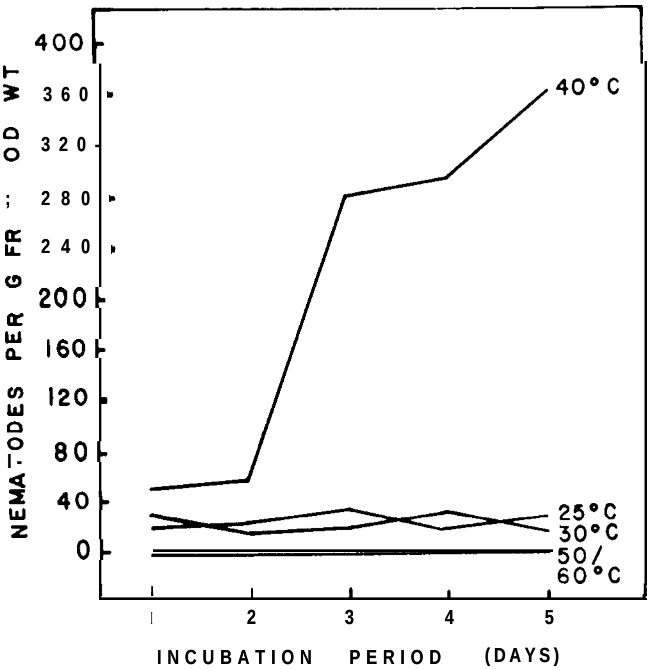

Figure 3.--Effect of moderate temperatures (25 to 60 °C) on pinewood nematode population development in southern pine chips over a 5-day incubation period.

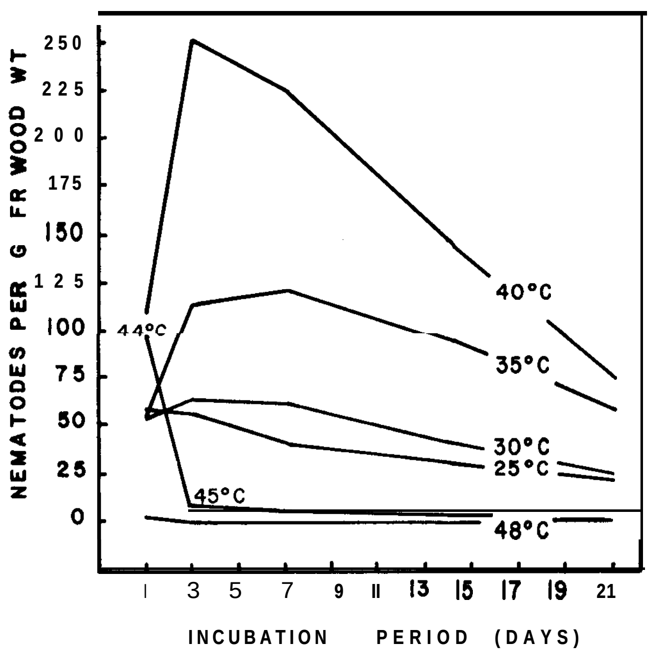

Figure 4.--Effect of moderate temperatures (25 to 48 °C) on pinewood nematode population development in southern pine chips over a Pl-day incubation period.

then declined to lower densities at later periods. After day 21, these densities approached those in the 35 °C treatment. The temperature of the incubator set at 44 °C crept to 45 °C between days 1 and 3. At day 1, the average nematode density in the 44 °C treatment was 96/g, but it declined to 6.2 nematodes/g by day 3 when the temperature had increased to 45 °C, and remained low until the end of the study. At 48 °C, the population density averaged 0.7 nematodes/g over time. The pinewood nematode increased significantly over time at temperatures between 35 and 44 °C in this study, with optimum for nematode reproduction in the 40 °C treatment.

In the temperature experiment where the chips had been autoclaved and inoculated with the bluestain fungus prior to being infested with the pinewood nematode, the analysis of variance showed experimental variation was due to the main effects (time and temperature). Among all four incubation temperatures, the nematode population density declined at day 3, then returned to the base level by day 6 (table 2). The mean number of nematodes extracted over time was 89 nematodes/g at 35 °C, which was significantly (P=0.01) greater than the 59

Table 2.--Effect of temperature on population development of pinewood nematode in autoclaved chips inoculated with Ceratocystis sp. and pinewood nematode

Incubation period (days)	Temperature (°C) 25	30	35	40	Mean[a]
1	87	75	104	61	81 A
3	30	21	38	35	31 B
6	62	79	114	65	80 A
9	58	72	100	53	71 A
Mean[a]	59 n	62 n	89 m	54 n	

[a]Within temperature and incubation periods. means followed by the same letter do not differ significantly (P=0.01) according to Duncan's multiple range test.

Table 3.--Pinewood nematodes extracted from 10 lots of wood chips incubated at 25 and 40 °C for 4 days[a]

Lot number	Temperature 25 °C	40 °C	Change (40/25 °C)
	Nematodes/g fresh weight		
1	400	410	1.0
2	500	640	1.3
3	380	1.160	3.0
4	200	460	2.3
5	560	1.200	2.1
6	260	780	3.0
7	340	232	0.7
8	500	820	1.6
9	620	314	0.5
10	96	224	2.3
Mean	386[b]	624[b]	1.6

[a] For 50-g samples extracted for 18 to 20 hours at 25 °C.

[b] Means are significantly different (P=0.01) according to t-test for paired observations.

Table 4.--Effect of high temperature on pinewood nematode populations in wood chips

Time (hours)	Temperature (°C) 50 °C	60 °C
	Percent[b]	
(0)[a]	(100.0)	(100.0)
1	47.2	0.8
2	16.5	0.3
3	6.1	0.5
5	1.5	0
8	0.6	0
13	0	0
24	0	0

[a] The base population density at time 0 was 127 nematodes/g. Each treatment combination was replicated three times. Data based on 50-g samples of chips extracted for 18 to 20 hours at 20 °C.

[b] Populations expressed as percentage of initial (time 0) population density.

nematodes/g at 25 °C, the 62 nematodes/g at 30 °C, and the 54 nematodes/g at 40 °C. The optimum temperature for reproduction of nematodes in this study was 35 °C.

When samples of 10 lots of naturally infested wood chips were incubated at 25 and 40 °C for 4 days, there was noticeable lot-to-lot variation in response to temperature (table 3). In lot 3, for example, there was a threefold increase in nematode levels between 25 and 40 °C. In lot 9, however, the densities were higher at 25 °C than at 40 °C. Nevertheless, 10 lots were significantly different (P=0.01), and the average increase in the population density between 25 and 40 °C was 1.6 times.

The pinewood nematode tolerated temperatures greater than 50 °C for no more than a few hours (table 4). After 1 hour at 50 °C, the nematode population density was 47 percent of the base level. After 13 hours, no pinewood nematodes were recovered from chip samples. After 1 hour at 60 °C, the population density was reduced by 99.8 percent, compared with the base level, and after 5 hours, no nematodes were recovered from chip samples.

In a low temperature experiment, the analysis of variance showed the experimental variation was attributed to the temperature x time interaction (P=0.01) (fig. 5). Nematodes survived in wood chips for only 3 days at -20 °C. The population densities were fairly constant over time at 0, 5, and 10 °C. At 15 °C, there was an increase in nematode densities at days 3 and 7, followed by a gradual decrease to day 14.

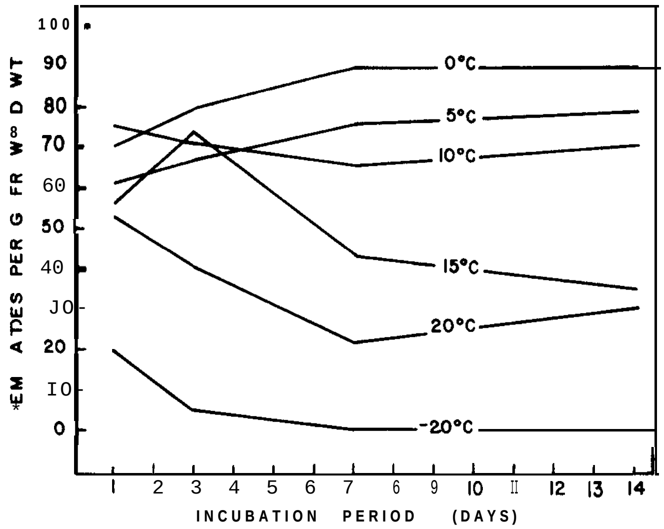

Figure 5.--Effect of low temperatures on pinewood nematode population development in southern pine chips.

Bluestain. Pinewood nematodes were recovered from 92 percent of the 38 lots sampled from July 1-5, 1985. The mean percentage by weight of wood chips with bluestain was 45 percent. The mean number of nematodes/g was 107 for chips with bluestain. This number was significantly (P=0.01) greater than the 82 nematodes/g for chips without bluestain.

When 10 lots of chips were divided into samples with or without bluestain and incubated at 40 °C for 6 days, the analysis of variance found that experimental variation was due to lots. The mean number of 669 nematodes/g extracted was somewhat higher than the 624 nematodes/g extracted in a previous study (see table 2). Based on a t-test for paired observations, there were significantly (P=0.01) more nematodes in the bluestain chips (747 nematodes/g) than in chips without bluestain (591 nematodes/g). These population densities are approximately seven times those at 40 °C in a previous comparison (107 and 82 nematodes/g, respectively). This result indicates that the effect of temperature was additive.

Chip moisture. Wood moisture markedly affected the population densities of nematodes in pine chips (fig. 6). After 5 days, the nematode densities in the chips declined as the percentage of moisture in the chips declined. In the control, the nematode level in the chips declined from 40 nematodes/g to 26 nematodes/g as the percentage of moisture loss reached 22. The nematode level also decreased in chips incubated over water.

Anaerobic environment. In the first study of pinewood nematode in an anaerobic environment, the population density was 157 nematodes/g for chips in an aerobic environment at 38 °C after 3 days. Under anaerobic conditions under the same temperature after 3 days, the population level was eight nematodes/g.

In the second experiment, the base (time 0) mean nematode density was 10 nematodes/g for the 10 chip samples. After 5 and 8 days at 38 °C in an aerobic environment, the population level was 43 and 40 nematodes/g, respectively. When chip samples were incubated for 5 days in an aerobic environment and then transferred to anaerobic conditions for 3 days, the population density dropped to 4 nematodes/g.

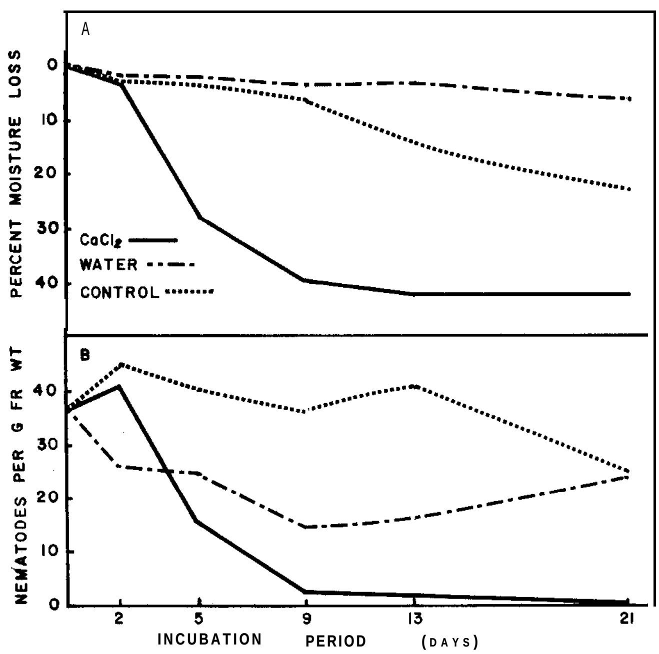

Figure 6.--Curves showing moisture loss in southern pine chips (A) and corresponding changes in population levels of pinewood nematode (B) at 35 °C over time.

Chip piles. There were significantly (P=0.01) fewer pinewood nematodes (8 nematodes/g) in the samples incubated in the chip pile covered with plastic than in samples from the uncovered pile (30 nematodes/g). In the covered chip pile, the highest nematode density was found in the samples placed in the shell of the lower or first level. The temperature at this location averaged 41 °C and never exceeded 45 °C. For the rest of the pile, the temperature averaged 43.9 °C and exceeded 45 °C for 60 to 366 hours, depending on the location of the probe. In the uncovered pile, the highest nematode density (52 nematodes/g) was in the center sample of the second layer. The temperature of the uncovered pile averaged 30.2 °C for the five probes. The mean ambient temperature was 30.2 °C. The temperature increase in the covered pile was largely due to solar radiation.

Pathogenicity. In the pathogenicity study, 20 percent of the slash pine seedlings inoculated with 20 (10 chip lots x 2 temperatures) populations of the pinewood nematode died within 16 weeks. There was no significant difference, according to t-test for paired observations, between the nematode gopulations from chips incubated at 25 C (22 percent mortality) and 40 °C (18 percent mortality). There was no lot-to-lot variation. None of the control seedlings died. Pinewood nematodes were the only nematodes recovered from symptomatic seedlings.

Discussion

Nematodes, particularly Rhabditis spp. and other saprobic nematodes, are commonly found in wood-chip piles. The pinewood nematode, a facultative parasite, was recovered from 92 percent of the chip samples collected from shipments from suppliers to the Savannah terminal in July. Since the living cells in fresh sapwood chips retain their viability only about 2 weeks at 21 °C (Feist and others 1971), the pinewood nematode is largely dependent on fungi in chips for nutrition and lives saprophytically in these chips for longer periods.

The complex thermodynamics of piled chips governs the population dynamics of the pinewood and other nematodes associated with southern pine chips. The spontaneous heating of the interior chips caused by oxidation is sufficient to kill all nematodes. The pinewood nematode is found in fresh chips and other chips that have not been subjected to this "self-pasturizing." The population densities of saprobic nematodes increased rapidly to high densities when interior chips were mixed with shell chips. This increase was probably caused by the reduction of predators and nematophagous fungi in the interior chips. Three nematophagous fungal species, including Arthobotrys oligospora Fres., have been cultured from the sapwood of slash pine (Esser and others 1983). Because of the spontaneous heating in piled chips, the pinewood nematode densities in chip piles should normally decrease to low levels over time.

In the present study, the optimum temperature range for the reproduction of the pinewood nematode in southern pine chips was 35 to 40 °C. These values are considerably higher than those in other reports. Most studies on the effects of temperature on the population dynamics of the pinewood nematode have been done on Botrytis cinerea in culture. For example, the optimum temperature for the pinewood nematode on B. cinerea has been reported as 25 °C (Dozono and Yoshida 1974). Mamiya (1983,1984) reports that the pinewood nematode will not reproduce at over 33 °C. Temperature studies using mesophilic fungi as a host for the pinewood nematode failed to consider the effect of temperature on the growth and physiology of the fungus. In chips, for example, over 200 species of fungi have been isolated, including such common thermotolerant fungi as Phanerochaete chrysosporium Burdsall & Eslyn and Aspergillus fumigatus Fres., which grow fastest at 35 to 40 °C (Bergman 1985; Burdsall and Eslyn 1974; Hulme 1979). These thermotolerant fungi may be a suitable food source for the pinewood nematode and explain why the nematode reproduces well at this temperature range in wood chips,.

Although the optimum temperature range of 35 to 40 °C was noted in several experiments, one study indicated that the pinewood nematode populations in different lots of southern pine chips did not always respond in the same manner to incubation at 40 °C. In some lots, the chips were probably less than 2 weeks old and the pinewood nematodes were probably feeding on the epithelial cells of the resin ducts (phytophagous). In other lots where the nematodes were mycophagous, reproduction was likely influenced by the presence or absence of thermotolerant fungi.

At temperatures above 45 °C, the population densities of the nematode in pine chips rapidly declines. To illustrate this temperature x time relationship, it takes about 13 hours for the population to decline to zero at 50 °C but only 1 hour at 60 °C. Unfortunately, it may not be economically feasible to use such a high temperature to control the pinewood nematode in wood chips because of the limited heat conductivity of wood and the rate the chips are loaded on ships (600 to 700 t/h). One estimate suggests it would take over 33 million Btu's/h to raise 10 t of wet wood chips/min 20 °C (W.E. Buske, Wolverine Corp.; personal communication). The energy costs and high associated capital investment have ruled this approach out for now.

The presence of bluestain in chips is an indicator of infestation of pines by bark beetles, but it cannot be used accurately to quantify nematode infestation of shipments from suppliers. Because of the frequent association of sawyers with southern pine (*Dendroctonus frontalis* Zimm.) and engraver (*Ips calligraphus* (Germ.) beetles (Coulson and others 1976; Miller 1984), shipments with bluestained chips frequently contained pinewood nematodes. Quite often, however, lots with a high percentage of chips with bluestain failed to yield nematodes. Pinewood nematodes have also been recovered from samples in which there are few chips with bluestain, if any.

Fresh chips with bluestain yielded significantly more nematodes than those without bluestain. The magnitude of the difference (1.3 times), however, was not as great as might have been expected for this mycophagous organism. Other fungi that colonize chips or wood of trees attacked by sawyers and bark beetles (Bergman 1985; Burdsall and Eslyn 1974; Luzzi and others 1984; McCrawley and others 1980) may prove to be more important in the ecology of pinewood nematodes than *Ceratocystis* spp. *Gliocladium virens* Miller (McCrawley and others 1985), for example, has been isolated from nematode-infested slash pines and is an excellent host for pinewood nematodes. Because of the thermogenic environment of piled wood chips, the role of thermotolerant fungi, such as *Phanerochaete chrysosporium* and *Aspergillus fumigatus*, in the development of pinewood nematodes needs to be investigated.

Pinewood nematodes are intolerant of anaerobic conditions at 38 °C. Since they are aerobic or semi-aerobic invertebrates, fumigants such as phosphine may be effective in controlling nematodes in wood chips stored in the holds of ocean vessels. Preliminary tests with phosphine show promise for controlling the nematode in wood chips (J.G. Leesch, ARS Stored Product Insects Research and Development Laboratory, Savannah, GA.; personal communication).

It is doubtful whether southern pine chips could serve as a source of inoculum for the establishment of pine wilt disease in countries that import them. Unless the nematode is transmitted by a vector, it is destined to be digested in the pulping process. The basic biological requirements of pine sawyers cannot be met by wood chips (Webb 1909; Hellrigl 1971).

The author thanks Bergitta Kemp and Wayne Stubbs of Savannah Sales, Inc., and the staff at Woodchip Export Company for assistance in this study.

The study was partially funded by a grant from the Georgia Forestry Commission.

Literature Cited

Bergman, Östen. Deterioration and protection of pulpwood chips in outdoor storage (Nedbrytning och skydd av ❑ assavedsflis vid lagring utomhus). Rep. 170. Uppsala. Sweden: Swedish University of Agricultural Science, Department of Forest Products; 1985. 95 pp.

Burdsall. H.H., Jr.; Eslyn. W.E. A new Phanerochaete with a Chrysosporium imperfect stage. Mycotaxon 1:123-133; 1974.

Coulson. R.N.; Mayyasi, A.M.; Foltz, J.L.; Hain, P.P. Interspecific competition between Monochamus titillator and Dendroctonus frontalis. Environmental Entomology 5:235-247; 1976.

Dozono, Yasou; Yoshida, Nariaki. Application of logistic curve for the population growth of pine wood nematode. Bursephelenchus lignicolus. on the cultures of Botrytis cinerea. Journal of the Japanese Forestry Society 56:146-148; 1974.

Dwinell. L. David; Barrows-Bronddus. Jane. Pine wilt and pitch canker of Virginia pine in seed orchards. In: Proceedings of the 17th Southern Forest Tree Improvement Conference; 1983 June 7-9: Athens, GA. Southern Forest Tree Improvement Committee sponsored publication 39; 1983:55-62.

Esser, R.P.; Wilkinson, R.C., Jr.; Harkcom, K.J. Pinewood nematode (Bursaphelenchus xylophilus) survey in Florida. Proceedings of the Soil and Crop Science Society of Florida. 42:127-132; 1983.

Feist, W.C.; Springer, E.L.; Uajny. C.J. Viability of parenchyma cells in stored green wood. Tappi 54:1295-1297; 1971.

Hellrigl, K.G. Die Bionomie der europaischen Monochamus-Arten (Coleopt.. Cerambycid) und ihre Bedeutung fiir die Forst- und Holzwirtschaft. Bolzano, Italy: Centro di Entomologia Alpina e Porestale del Consiglio Nazionale delle Richerche; Publlicazione N. 154; 1971:367-509.

Hulme, M.A. Outside chip storage: dctcriorntion during outside chip storage. In: Hatton, J.V., ed. Chip Quality Monograph. Pulp and Paper Technol. Ser. 5: 1979:213-244.

Kable. P.F.; Mai, W.P. Influencc of soil moisture on Pratylenchus penetrans. Nematologica 14:101-122; 1968.

Luzzi, M.A.; Wilkinson, R.C.. Jr.: Tarjan, A.C. Transmission of the pinewood nematode, Bursaphelenchus xylophilus. to slash pine trees and log bolts by a cerambycid beetle, Monochamus titillator. in Florida. Journal of Nematology 16:37-40; 1984.

Mamiya, Y. Pathology of the pine wilt disease caused by Bursaphelenchus xylophilus. Annual Review of Phytopathology 21:201-220; 1983.

Mamiya, Y. The pine wood nematode. In: Nickle, W.R., ed. 1984. Plant and insect nematodes. New York: Marcel Dckker. Inc.; 1984:589-625.

Massey, Calvin L. Biology and taxonomy of nematode parasites and associates of bark bcctlcs in the United states. Agric. Handb. 446. Washington, DC: U.S. Depnrtmcnt of Agricullurc. Forest Service; 1974. 233 pp.

McCrawley, E.C.; Jones, J.P.; Birchfield, W. Reproduction of Bursaphelenchus lignicolus on fungi isolated from B. lignicolus-infested pine trees. Journal of Ncmotology 12:231; 1980. Abstract.

McCrawley, E.C.; Winchcll. K.L.; Jones, J.P. [and others]. Population dcvclopmcnt and influence of Bursaphclcnchus xylophilus on Gliocladium virens. Journal of Nemntology 17:69-76; 1985.

Miller, M.C. Effect of exclusion of insect associates on Ips calligrnphus (Germ.) (Coleoptera: Scolylidac) broad emergence. Zeitschrift für Angewandte Entomologie 97:298-304; 1984.

Rothrock. C.W.. Jr.; Smith, W.R.: Lingren, R.M. The effects of outside storage on slash pine chips in the South. Tappi 44: 65-73; 1961.

Riihm. W. Die Ncmntodcn der Ipidcn. Parasitologishe Schriftcnreischc 6: 1956. 437 pp.

Saucier. J.R.; Miller. R.L. Detcriorntion of southern pine chips during summer and winter storage. Forest Products Journal 11:371-379; 1961.

Schmidt, P.L. Observations on spontaneous heating toward combustion of commercial chip piles. Tappi 52:1700-1701; 1969.

Springer. E.L.; Haljny, G.J. Spontaneous heating in piled wood chips. I. Initial mechanism. Tappi 53:85-86; 1970.

Tarjan, A.C.; Aragón, Carlos Baeza. An analysis of the genus Bursaphelenchus Fuchs, 1937. Nematropica 12:121-144; 1982.

Webb, J.L. The southern pine sawyer. Bull. 58. Washington, DC: U.S. Department of Agriculture. Bureau of Entomology; 1909:41-56.

Wingfield. M.J. Transmission of pinewood nematode to cut timber and girdled trees. Plant Disease 67(1):35-37; 1983.

Wingfield. M.J.; Blanchette. R.A.; Nicholls. T.M.; Robbins, K. The pine wood nematode: a comparison of the situation in the United States and Japan. Canadian Journal of Forest Research 12:71-74; 1982.

Dwinell. L. David

Ecology of the pinewood nematode in southern pine chip piles. Res. Pap. SE-258. Asheville, NC: U.S. Department of Agriculture. Forest Service. Southeastern Forest Experiment Station; 1986. 14 pp.

The optimum temperature range for pinewood nematodes in southern pine chips was 35 to 40°C. Nematode populations declined at temperatures of -20 $^{\circ}$C. at temperatures above 45 $^{\circ}$C. and in anaerobic environments. Wood moisture content and presence of bluestain fungus also influenced nematode populations.

KEYWORDS: Bursaphelenchus xylophilus. temperature. bluestain. pine wilt. slash pine, loblolly pine, pathogen.

Dwinell. L. David

Ecology of the pinewood nematode in southern pine chip piles. Res. Pap. SE-258. Asheville. NC: U.S. Department of Agriculture. Forest Service. Southeastern Forest Experiment Station: 1986. 14 PP.

The optimum temperature range for pinewood nematodes in southern pine chips was 35 to 40°C. Nematode populations declined at temperatures of -20 $^{\circ}$C. at temperatures above 45 $^{\circ}$C. and in anaerobic environments. Wood moisture content and presence of bluestain fungus also influenced nematode populations.

KEYWORDS: Bursaphelenchus rylophilus. temperature. bluestain. Pine wilt, slash pine. loblolly pine, pathogen.